EFFECT OF TITANIUM SURFACE ROUGHNESS ON CELL ADHESION OF HUMAN OSTEOBLAST-LIKE CELLS (MG63)

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Statement of problem. The effects of surface roughness have not or insufficiently been analyzed on earlier events such as cell adhesion though cell behavior most germane to implant performance is cell adhesion.

Purpose. The purpose of this study was to evaluate cell adhesion of osteoblast-like cells (MG63) onto three types of titanium disks with varying roughness using the Elisa assay.

Materials and methods. Representative disks from each group (SLA, HA, machined) were subjected to surface analysis and surface roughness was measured by the optical interferometer (Accura 2000, Intekplus Co., Seoul, Korea). Following this, MG63 cells were cultured on the titanium disks and released. Cell adhesion measurements using the Elisa assay were performed specifically at three points: after 24, 48, and 72 hours of culture.

Results. Among the 3 types of surface analyzed, the SLA surface was the roughest with a Ra value of 1.114 μm followed by HA coated surface and machined surface, consecutively. The optical density values for the SLA surface group was significantly higher than that of the machined and HA coated suface groups following 24 and 48 hours of culture. The cell culture on HA coated surface showed significantly higher values compared to the machined surface following 24, 48 and 72 hours of culture.

Conclusion. The results suggest that surface treatment of titanium surfaces enhanced cell adhesion of human osteoblast-like cells (MG63).

Key Words
Cell adhesion, Osteoblast-like cells (MG63), Elisa assay, Optical density values

Since the introduction of the concept of osseointegration, implants of various composition and design have been studied and developed. Currently, commercially pure titanium (Ti) is the material of choice for uncoated implants because of its biologic acceptance in bone.[1,2] This high degree of biocompatibility is thought to result, in part, from the oxide layer that resides over the titanium surface and facilitates the bonding of

the extracellular matrix at the implant-tissue interface.[3,4]

The characteristic compositon and structure of the oxide layer may be altered when different preparation techniques are applied to the surface of the titanium. It has been shown that methods of implant surface preparation can significantly affect the resultant properties of the surface and subsequently the biological responses that occur at the surface. Windeler et al.[5] demonstrated that osteoblast-like cells adhered more strongly to Titanium surfaces whereas osteoclasts adhered more strongly to hydroxyapatite surfaces. In a study comparing cell adhesion to different surfaces, Martin et al.[6] have found that osteoblast-like cells adhered more strongly to Titanium surfaces than they do to smooth surfaces. Moreover, it was also shown that surface roughness affects proliferation; as roughness increases, proliferation decreases.

MG63 cells, an osteoblast-like human cell line, have phenotypic and genetic characteristics typical of a relatively immature osteoblast.[7] Although MG63 cells do not calcify their extracellular matrix in culture, the cell line is sufficiently differentiated far along the osteogenic lineage to serve as an excellent system for examining early events in the response of bone cells to surfaces. Studies using these cells have demonstrated positive effects of increased surface roughness of titanium discs on cellular differentiation and matrix production.[6]

However, these effects of surface roughness have not or insufficiently been analyzed on earlier events such as cell adhesion though cell behavior most germane to implant performance is cell adhesion. The objective of this study was to evaluate cell adhesion of osteoblast-like cells (MG63) onto three types of Titanium disks with varying roughness using the Elisa assay.

MATERIALS AND METHODS

Surface preparation

The Titanium disks subjected for cell culture were processed to produce three types of surfaces of varying roughness as follows.

1. Machined
2. SLA surface: Disks were blasted with 220um corundum grit at 3 bar until the surface reached a uniform gray tone. They were then acid-etched in hydrochloric acid/sulfuric acid at room temperature for 4 minutes followed by rinsing in deionized water, neutralization in 5 % sodium bicarbonate solution, and three 5-minute rinses in deionized water contained in the ultrasonic bath.
3. HA: Thin HA layers were deposited on Titanium-substrates by an electron beam deposition method. After evacuating the chamber down to 10^{-7} torr using a cryopump (OB-10, Helix Technology, Mansfield, MA, USA), an electron beam (Telemark, Fremont, CA, USA) of 8.5 kV and ~0.1 A was directed onto the source target. Prior to deposition, the substrates were sputter-cleaned with an ion beam (Mark II, Commonwealth Scientific, Alexandria VA, USA) of 120 V and 0.6 A for 20 minutes. In order to increase the uniformity of the coating layer during the deposition process, substrates were rotated at a speed of 8 rpm. The targets were made using a commercially available HA ($Ca_{10}(PO_4)_6(OH)_2$) powder (Alfa Aesar Co., Ward Hill, MA, USA). Extra weight % of CaO powder (Cerac Co., Milwaukee, WI, USA) was added to the HA and mixed by ball milling in ethyl alcohol for 24 hours with Al_2O_3 balls as media. The powder mixtures were sintered in air at 1200° C for 2 hours. The deposited coating layer was heat treated in air at temperatures between 300° C and 500° C for 1 hour.

Table I. Surface roughness (Ra) of the titanium implants (Mean±SD, n=15)

	Ra (arithmetic mean of the absolute values of the surface)
Machined	0.45±0.04 μm[a]
HA coated	0.49±0.06 μm[a]
SLA	1.11±0.08 μm[b]

* The same letter denotes groups that were not significantly different from each other (P>0.05)

Surface analyses

Representative 3 disks from each group were subjected to surface analysis. 5 different areas of each samples were measured. Surface roughness was measured by optical interferometer (Accura 2000, Intekplus Co., Seoul, Korea). (Fig. 1, 2 & 3) This system provides visual images as well as numerical values for the different surface roughness parameters.

Cell cultures

MG63 osteoblast-like cells, originally isolated from a human osteosarcoma, were used in this study. This osteoblast-like cell line has been well-characterized and contains numerous osteoblastic traits that are typical of a relatively immature osteoblast, including high levels of 1,25-$(OH)_2D_3$-responsive alkaline phosphatase and osteocalcin synthesis inhibition of proliferation when treated with 1,25-$(OH)_2D_3$. As a result, they are a good model for examining the early stages of osteoblastic differentiation. MG63 cells were obtained from the American Type Culture Collection (Rockvill, MD). Cells were plated at a concentration of $2.5x10^5$/ml in Dulbecco' s modified Eagel' s medium (DMEM) containing 10% fetal bovine serum (FBS) and 1 % antibiotics and cultured at 37° in 5% CO_2.

Cell adhesion assay (ELISA ASSAY)

Cell adhesion onto each titanium disk was measured using the Elisa assay. 8 disks were used for each groups. After cell culture, cells were washed in PBS (Phosphated buffered saline) and fixed with 10% formalin for 15 minutes. Cells were then stained overnight with 1% crystal violet 1ml. After staning, disks containing the cultured cells were washed 3 times with DW. With the addition of 1% SDS (sodium dodesil sulfate) 0.2ml and vortexing for 5 minutes, cells from the disk surface were released. Lysed cells were transferred to 96-well plate and the absorption optical density was calculated at 570nm using the Elisa assay.

RESULTS

Surface analysis

The results of the optical interferometer analysis are shown in Table I. Among the 3 types of surface analyzed, the SLA surface was the roughest with a Ra value of 1.114 μm followed by HA coated surface and machined surface, consecutively. (Fig. 1, 2 & 3)

Cell adhesion assay

Cell adhesion was measured using the Elisa assay following 24, 48, and 72 hours of culture. The control sample was calculated at the beginning of each cell culture and showed no significant difference in absorption optical density values between the samples. The optical density values for the SLA surface group was significantly higher than that of the machined and HA coated suface groups following 24 hours of culture. This was also true following 48 hours of culture as the SLA surface displayed significantly higher values. The cell culture on HA coated surface showed significantly higher values compared to the machined surface following 24, 48 and 72 hours of culture. All three groups showed decreased optical density values following 72 hours of culture. The results of the Elisa test are listed in Table II.

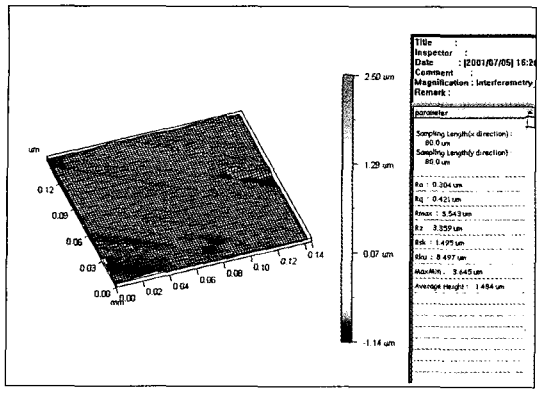

Fig. 1. Three-dimensional image analysis of the machined surface measured by the optical interferometer.

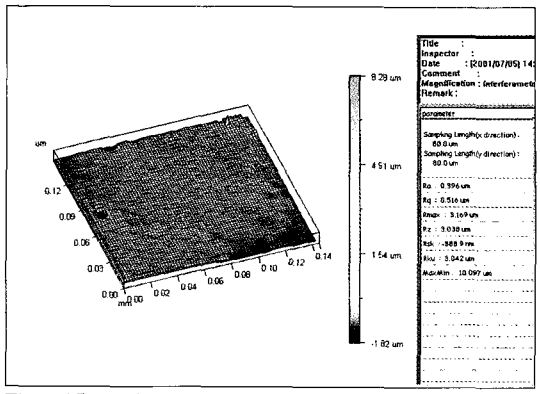

Fig. 2. Three-dimensional image analysis of the SLA surface measured by the optical interferometer.

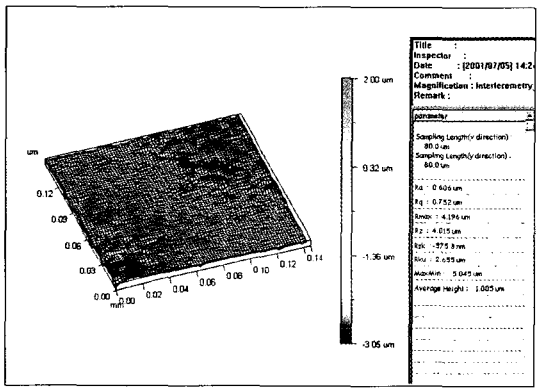

Fig. 3. Three-dimensional image analysis of the HA coated surface measured by the optical interferometer.

Table II. Results of the Elisa test (n=8)

	Control	24 hours	48 hours	72 hours
machined	0.16 ± 0.04^a	0.49 ± 0.03^a	0.49 ± 0.05^a	0.35 ± 0.04^c
HA coated	0.09 ± 0.03^a	0.66 ± 0.02^b	0.6 ± 0.02^b	0.08 ± 0.02^a
SLA	0.1 ± 0.03^a	1.1 ± 0.05^c	1.43 ± 0.06^c	0.19 ± 0.03^b

*The same letter denotes groups that were not significantly different from each other (P>0.05)

DISCUSSION

The objective of this study was to evaluate cell adhesion of osteoblast-like cells (MG63) onto three types of Titanium disks with varying roughness using the Elisa assay. Representative disks from each group was first subjected to surface roughness analysis using the optical interferometer. (Accura 2000, Intekplus Co., Seoul, Korea). The results revealed that the Ra values of SLA surface were higher than that of HA coated and machined surfaces. Also, the Ra values of HA coated implants when compared to machined implants, showed only a marginal difference. This was interesting in respect to earlier findings where HA coated implants consistently showed rougher surfaces than the machined implants. Not well understood, one may only speculate the cause of these results because direct comparisons to earlier studies are difficult to make since different methodology and techniques have been applied during processing of these surfaces. Different preparation techniques may have contributed to this phenomenon.

As the second part of this study, cell adhesion was measured following 24, 48 and 72 hours of culture. The control sample was calculated at the beginning of each cell culture. The results of the cell adhesion assay suggest enhanced adhesion of osteoblastic cells on surface treated

titanium disks. Also, as roughness increased, so did adhesion of osteoblastic cells. This is in accordance with a number of studies that evaluated the effect of surface roughness on early cellular activities of human osteoblasts or osteoblastic cells. Bowers et al.[8] have demonstrated that rough titanium surfaces which measured in the micrometer range due to sandblasting or coating by plasma spray significantly enhanced cellular attachment and the production of extracellular matrix and subsequent mineralization in vitro. Also, it was shown by Michaels et al.[16] that osteoblast-like cells exhibit greater initial attachment to rough Titanium surfaces.

Results following 72 hours of culture suggest decrease in optical density values in all three types of surfaces. This may be due to the fact that cell adhesion properties of osteoblastic cells occur early, presumably within 24 hours of culture. Further evaluation is needed to confirm this phenomenon.

The present study possessed interesting aspects as it utilized (MG63) osteoblast-like cells and Elisa assay for evaluation. In a previous study using human osteoblast-like cell line (MG63), Martin et al.[6] showed that increasing surface roughness of titanium discs reduced proliferation but induced cellular differentiation and matrix production. However, earlier events such as cell adhesion was not evaluated. This study confirmed that increasing surface roughness contributes to adhesion of osteoblast-like cells (MG63). Although further studies seem necessary to strengthen this hypothesis, this finding presents a significant leap in understanding the early events of osteoblast-like cells on Titanium surfaces.

CONCLUSION

The effects of surface roughness have not or insufficiently been analyzed on earlier events such as cell adhesion though cell behavior most germane to implant performance is cell adhesion. The purpose of this study was to evaluate cell adhesion of osteoblast-like cells (MG63) onto three types of titanium disks with varying roughness. Representative disks from each group (SLA, HA, machined) were subjected to surface analysis and surface roughness was measured by the optical interferometer (Accura 2000, Intekplus Co., Seoul, Korea). Following this, MG63 cells were cultured on the titanium disks and released. Cell adhesion measurements using the Elisa assay were performed specifically at three points: after 24, 48 and 72 hours of culture. It can be concluded that :

1. The surface roughness of machind group was not different from that of HA coated group. ($P>0.05$) The SLA group showed the roughest surface among three groups.
2. The SLA group showed the highest cell adhesion following the HA group and the machined group. ($P<0.05$) After 24 hours and 48 hours.
3. Cell attachment of all three groups decreased following 72 hours of culture.
4. Surface modifications using HAcoating and SLA can promote cellular attachments and enhance bone formation.

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